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(54) Title: **CIS/TRANS RIBOREGULATORS**

(57) **Abstract:** The present invention provides nucleic acid molecules, DNA constructs, plasmids, and methods for post-transcriptional regulation of gene expression using RNA molecules to both repress and activate translation of an open reading frame. Repression of gene expression is achieved through the presence of a regulatory nucleic acid element (the cis-repressive RNA or crRNA) within the 5' untranslated region (5' UTR) of an mRNA molecule. The nucleic acid element forms a hairpin (stem/loop) structure through complementary base pairing. The hairpin blocks access to the mRNA transcript by the ribosome, thereby preventing translation. In particular, in embodiments of the invention designed to operate in prokaryotic cells, the stem of the hairpin secondary structure sequesters the ribosome binding site (RBS). In embodiments of the invention designed to operate in eukaryotic cells, the stem of the hairpin is positioned upstream of the start codon, anywhere within the 5' UTR of an mRNA. A small RNA (trans-activating RNA, or taRNA), expressed in trans, interacts with the crRNA and alters the hairpin structure. This alteration allows the ribosome to gain access to the region of the transcript upstream of the start codon, thereby activating transcription from its previously repressed state.

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# INTERNATIONAL SEARCH REPORT

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## A. CLASSIFICATION OF SUBJECT MATTER

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US CL : 536/24.1, 24.5; 800/13; 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.1, 24.5; 800/13; 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,272,065 A (INOUE et al.) 21 December 1993 (21.12.93), see entire document especially Figure 3 and the caption thereto.	1-37,52,61,108,112
Y		38-40,53-60,110,111,114,115
A	US 5,514,546 A (KOOL) 7 May 1996 (07.05.96), see entire document.	1-242
A	WO 99/10487 A2 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 4 March 1999 (04.03.99), see entire document.	1-242

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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# INTERNATIONAL SEARCH REPORT

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**Continuation of B. FIELDS SEARCHED Item 3:**  
**EAST, STN (MEDLINE, BIOSIS , CAPLUS, EMBASE, CANCERLIT)**  
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